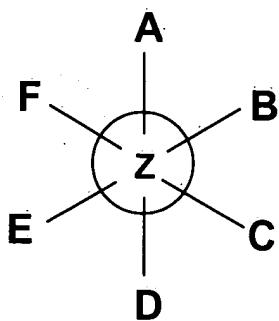


WE CLAIM:

1. A composition of matter comprising a reporter compound, the reporter compound having a four-, five-, or six-member aromatic ring Z, with substituents A, B, C, D, E, and F, according to the formula:
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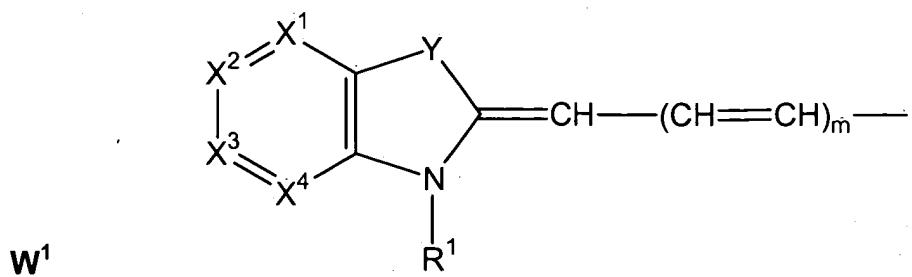


- wherein F is absent when Z is a five-member ring, and wherein E and F are absent
10 when Z is a four-member ring;

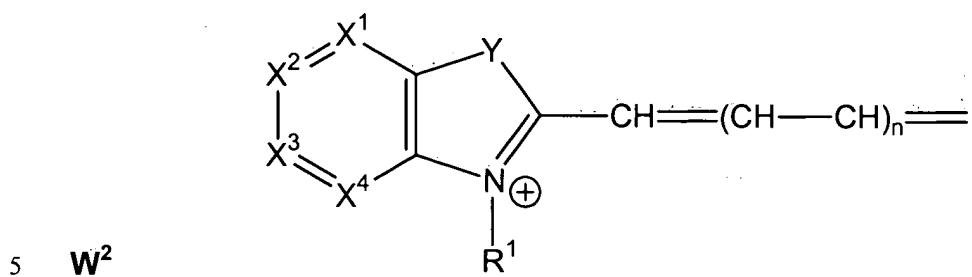
wherein A, B, C, D, E, and F may be present in any order, provided that B and C are adjacent, in which case each of A, D, E, and F is neutral, or provided that B and C are separated by one of A, D, E, or F, in which case one of A, D, E, and F is negatively charged;

- 15 when the A substituent is neutral, A is selected from the group consisting of =N-R^c, wherein R^c is selected from the group consisting of aliphatic, heteroatom-substituted aliphatic, polyether, aromatic, reactive aliphatic, and reactive aromatic groups; when the A substituent is negatively charged, A is -(N-R^c)⁻;

each B and C substituent is selected from the group consisting of W¹ and W²,
20 wherein W¹ and W² have the respective formulae



and

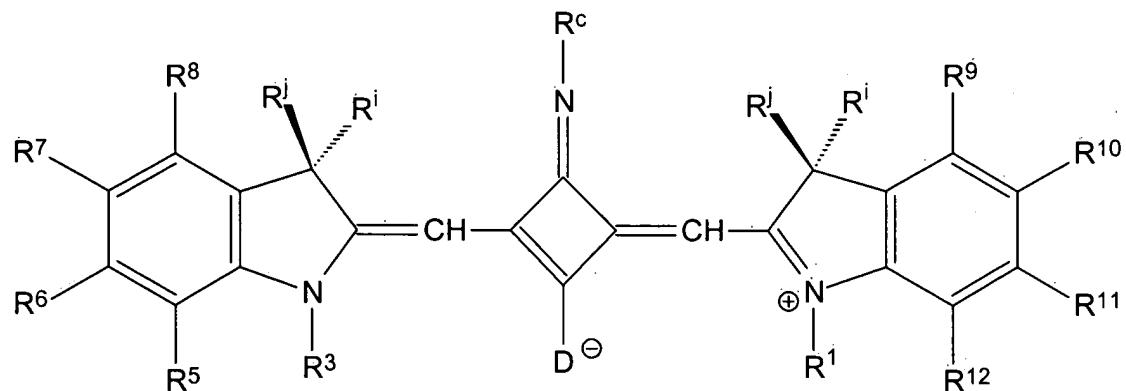


where each B and C substituent is W¹ if B and C are adjacent on Z, and one of B and C is W¹ and the other of B and C is W² if B and C are separated by one of A, D, E, and F on ring Z;

- 10 each D, E, and F substituent, when present and neutral, is independently selected from the group consisting of =O, =S, =Se, =Te, =N-R^c, and =C(R^f)(R^g), wherein R^c is selected from the group consisting of aliphatic, heteroatom-substituted aliphatic, polyether, aromatic, reactive aliphatic, and reactive aromatic groups, R^f and R^g being selected from the group consisting of carboxylic acid, cyano, carboxamide, carboxylic ester, and aliphatic amine groups; D, E, and F, when present and negatively charged, are independently selected from the group consisting of -O⁻, -S⁻, -Se⁻, -Te⁻, -(N-R^c)⁻, and -(C(R^f)(R^g))⁻;
- 15

- m and n are independently selected from the group consisting of 0, 1, and 2;
- Y is independently selected for each of B and C from the group consisting of O, S, Se, Te, N-R^h, and C(Rⁱ)(R^j), wherein R^h is selected from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, and reactive aliphatic groups, and
- 5 wherein each of Rⁱ and R^j is selected from the group consisting of aliphatic and reactive aliphatic groups;
- each R¹ is independently selected for each of B and C from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive
- 10 groups capable of covalent attachment to a carrier, and ionic substituents capable of increasing the hydrophilicity of the entire compound;
- each of X¹, X², X³, and X⁴ is independently selected for each of B and C from the group consisting of N, O, S, and C-R^k, wherein R^k is selected from the group consisting of H, F, Cl, Br, I, aliphatic groups, alicyclic groups, aromatic groups, linked carriers,
- 15 reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents capable of increasing the hydrophilicity of the entire compound, parts of a condensed aromatic or heterocyclic ring, and parts of a substituted condensed aromatic or heterocyclic ring; and
- 20 each H may be independently replaced by a fluorine.

2. The composition of claim 1, wherein the reporter compound has the formula



5 where D is -O or -S;

R¹ and R³ are independently H, -(CH₂)_k-L, or -(CF₂)_k-L where k = 1 – 30, and each L is one of H, F, Cl, Br, I, NH₂, SO₃⁻, COOH, and CO-NHS; and

R⁵ – R¹² are each independently H, F, or SO₃⁻.

R^c is selected from the group consisting of aliphatic, heteroatom-substituted aliphatic, polyether, aromatic, reactive aliphatic, and reactive aromatic groups, hydrogen, CN, SO₃H, and COO-R^m, where R^m is selected from a group consisting of hydrogen, aliphatic substituents, aromatic substituents, reactive aliphatic substituents, reactive aromatic or heterocyclic substituents, and linked carriers.

R^j and Rⁱ are H, aliphatic groups, alicyclic groups, aromatic groups, polyethers, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents and spacers containing one or more ionic substituents, capable of increasing the hydrophilicity of the entire compound; or R^j and Rⁱ taken in combination

form a ring-system that is optionally substituted by one or more reactive or ionic substituents.

3. The composition of claim 1, wherein Z is based on squaric acid, croconic acid, or rhodizonic acid.

4. The composition of claim 1, wherein at least one substituent of Z includes a reactive group.

10 5. The composition of claim 4, wherein the reactive group is selected for reacting with amine moieties from the group consisting of N-hydroxysuccinimide esters, isothiocyanates, and sulfonylhalogenides.

15 6. The composition of claim 4, wherein the reactive group is selected for reacting with thiol moieties from the group consisting of iodoacetamides and maleimides.

7. The composition of claim 4, wherein the reactive group is selected for reacting with nucleic acids from the group consisting of phosphoramidites.

20

8. The composition of claim 1, wherein at least one substituent of Z includes a linked carrier.

9. The composition of claim 8, wherein the carrier is selected from the group consisting of polypeptides, polynucleotides, beads, microplate well surfaces, and metallic nanoparticles.
- 5 10. The composition of claim 9, wherein the carrier is a polypeptide or a polynucleotide.
- 10 11. The composition of claim 1, further comprising a carrier, which is associated covalently with the reporter compound through reaction with a reactive group on at least one substituent of Z.
- 15 12. The composition of claim 1, wherein at least one substituent of Z is an ionic substituent capable of increasing the hydrophilicity of the entire photoluminescent compound.
13. The composition of claim 12, wherein the ionic substituent is selected from the group consisting of SO_3^- , COO^- , PO_3^{2-} , O-PO_3^{2-} , PO_3R^- , $\text{O-PO}_3\text{R}^-$ and $\text{N}(\text{R}')_3^+$, wherein R and R' are independently an aliphatic or aromatic moiety.
- 20 14. The composition of claim 1, wherein the substituents of Z are selected so that the reporter compound is electrically neutral, increasing its hydrophobicity.
15. The composition of claim 1, wherein R' is $(\text{CH}_2)_n\text{COOH}$ or $(\text{CH}_2)\text{NH}_2$.

16. The composition of claim 1, wherein the reporter compound is capable of covalently reacting with at least one of biological cells, DNA, lipids, nucleotides, polymers, proteins, and pharmacological agents.

5 17. The composition of claim 1, wherein the reporter compound is covalently or noncovalently associated with at least one of biological cells, DNA, lipids, nucleotides, polymers, proteins, and pharmacological agents.

18. The composition of claim 1, wherein m and n are 1.

10 19. The composition of claim 1, wherein B and C are adjacent, and are linked to Z through a 1,2 linkage.

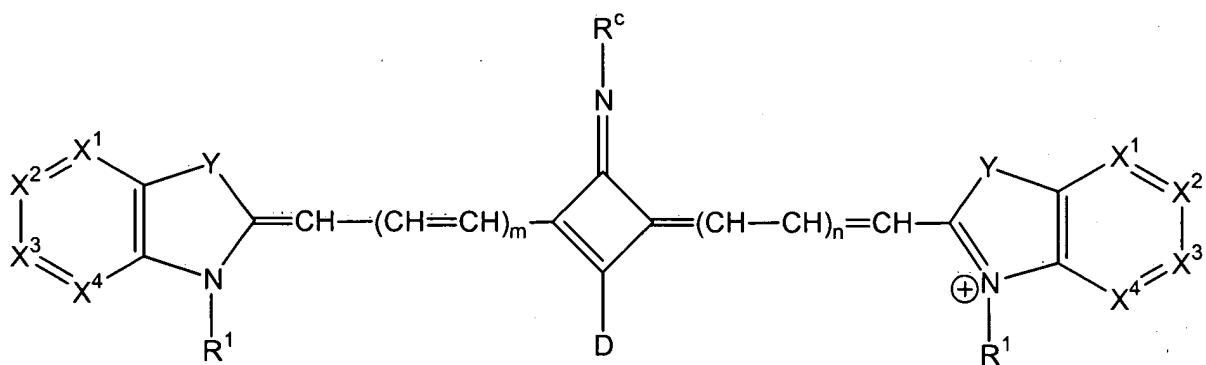
15 20. The composition of claim 1, wherein B and C are separated by one of A, D, E, or F, and are linked to Z through a 1,3 linkage.

21. The composition of claim 1, further comprising a second reporter compound selected from the group consisting of luminophores and chromophores.

22. The composition of claim 21, wherein one of the reporter compound and the second reporter compound is an energy transfer donor and the other is an energy transfer acceptor.

5 23. The composition of claim 1, wherein the reporter compound may be induced to luminesce by exposing the reporter compound to one or more of the following: electromagnetic energy, chemical energy, and electrochemical energy.

24. A reporter compound having the formula



wherein D is selected from the group consisting of O^- , S^- , Se^- , Te^- , $N-(R^c)^-$, and

5 $C(R^f)(R^g)^-$, where each R^c is selected from the group consisting of aliphatics, heteroatom-substituted aliphatics, polyethers, aromatics, reactive aliphatics, reactive aromatics, and linked carriers; R^f and R^g being selected from the group consisting of carboxylic acids, cyano, carboxamides, carboxylic esters, and aliphatic amines;

m and n are independently selected from the group consisting of 0, 1, and 2;

10 each Y is independently selected from the group consisting of O, S, Se, Te, N-
 R^h , and $C(R^i)(R^j)$, where R^h is selected from the group consisting of hydrogen, aliphatics, alicyclics, aromatics, and reactive aliphatics; and where each of R^i and R^j are H, aliphatic groups, alicyclic groups, aromatic groups, polyethers, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or
15 more reactive groups capable of covalent attachment to a carrier, ionic substituents and spacers containing one or more ionic substituents, capable of increasing the hydrophilicity of the entire compound; or R^i and R^j taken in combination for a ring-system that is optionally further substituted by one or more reactive or ionic substituents;

each R¹ is independently selected for each of B and C from the group consisting of hydrogen, aliphatics, alicyclics, aromatics, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, and ionic substituents capable of increasing the hydrophilicity of the entire compound;

each of X¹, X², X³, and X⁴ is independently selected from the group consisting of H, N, O, S, and C-R^k, wherein R^k is selected from the group consisting of H, F, Cl, Br, I, aliphatics, alicyclics, aromatics, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents capable of increasing the hydrophilicity of the entire compound, parts of a condensed aromatic or heterocyclic ring, and parts of a substituted condensed aromatic or heterocyclic ring; and

each hydrogen may be independently replaced by a fluorine.

25. The compound of claim 24, where R^c is selected from the group consisting of hydrogen, CN, OH, SO₃H, C=ONHR^m, COO-NHS and COO-R^m, where R^m is selected from a group consisting of hydrogen, aliphatic substituents, aromatic substituents, reactive aliphatic substituents, reactive aromatic substituents, and linked carriers.

26. A method of performing a photoluminescence assay, the method comprising:

- selecting a photoluminescent compound according to claim 1;
- exciting the photoluminescent compound with excitation light; and
- detecting emission light emitted by the photoluminescent compound.

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27. The method of claim 26, including the step of detecting fluorescence.

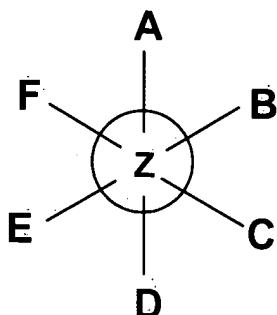
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28. The method of claim 26, including the step of detecting phosphorescence.

29. The method of claim 26, further comprising analyzing the emission light and determining at least one of luminescence intensity, lifetime, and polarization.

15
30. The method of claim 26, further comprising associating the photoluminescent compound with a second molecule.

31. The method of claim 26, further comprising performing multicolor multisequencing analysis based on *in-situ* hybridization.

32. A composition of matter comprising a photoluminescent compound, the photoluminescent compound having a four-, five-, or six-member aromatic ring Z, with substituents A, B, C, D, E, and F, according to the formula:



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wherein F is absent when Z is a five-member ring, and wherein E and F are absent when Z is a four-member ring;

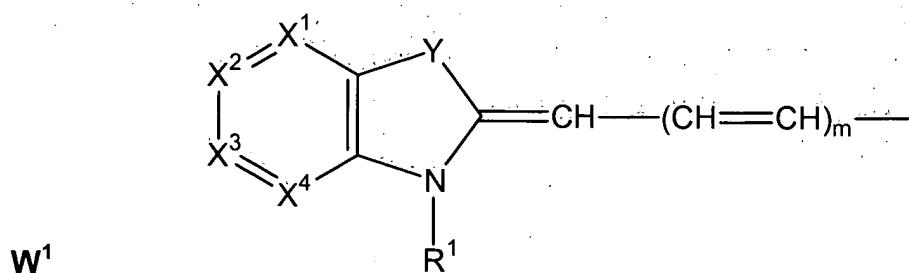
wherein A, B, C, D, E, and F may be present in any order, provided that B and C
10 are adjacent, in which case each of A, D, E, and F is neutral, or provided that B and C
are separated by one of A, D, E, or F, in which case one of A, D, E, and F is negatively
charged;

when the A substituent is neutral, A is =O; when the A substituent is negatively charged, A is -O⁻;

15 where each D, E, and F substituent, when present and neutral, is independently selected from the group consisting of =O, =S, =Se, =Te, =N-R^c, and =C(R^f)(R^g), wherein each of R^c is selected from the group consisting of aliphatic, heteroatom-substituted aliphatic, polyether, aromatic, reactive aliphatic, and reactive aromatic groups, hydrogen, CN, OH, SO₃H, and COO-R^m, where R^m is selected from a group consisting
20 of hydrogen, aliphatic substituents, aromatic substituents, reactive aliphatic

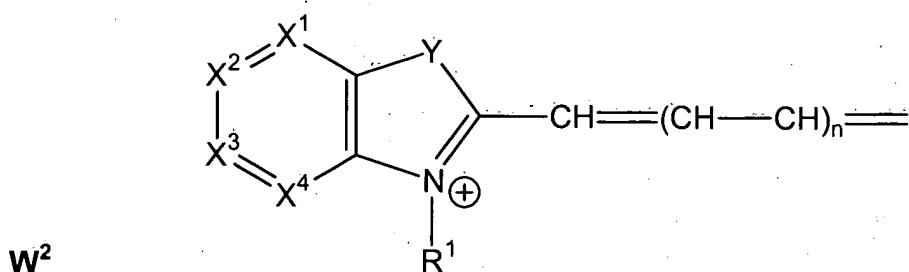
substituents, reactive aromatic substituents, and linked carriers, and R^f and R^g are selected from the group consisting of carboxylic acid, cyano, carboxamide, carboxylic ester, and aliphatic amine groups; D, E, and F, when present and negatively charged, are independently selected from the group consisting of -O⁻, -S⁻, -Se⁻, -Te⁻, -(N-R^c)⁻, 5 and -(C(R^f)(R^g))⁻;

each B and C substituent is selected from the group consisting of W¹ and W², wherein W¹ and W² have the respective formulae



10

and



15 where each B and C substituent is W¹ if B and C are adjacent on Z, and one of B and C is W¹ and the other of B and C is W² if B and C are separated by one of A, D, E, and F on ring Z;

m and n are independently selected from the group consisting of 0, 1, and 2; each Y is independently selected for each of B and C from the group consisting of O, S, N-R^h, and C(Rⁱ)(R^j), wherein R^h is selected from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, spacers bound to ionic and reactive groups, and Rⁱ and R^j are selected from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, polyether groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents and spacers containing one or more ionic substituents capable of increasing the hydrophilicity of the entire compound; or Rⁱ and R^j taken in combination form a ring-system that is optionally further substituted by one or more reactive or ionic substituents; provided that at least one Y is C(Rⁱ)(R^j), and at least one of Rⁱ or R^j includes a reactive group, a linked carrier, or an ionic substituent capable of increasing the hydrophilicity of the entire compound;

each R¹ is independently selected for each of B and C from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, polyether groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, and ionic substituents capable of increasing the hydrophilicity of the entire compound;

each of X¹, X², X³, and X⁴ is independently selected for each of B and C from the group consisting of N, O, S, and C-R^k, wherein R^k is selected from the group consisting of H, F, Cl, Br, I, aliphatic groups, alicyclic groups, aromatic groups, polyether groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic

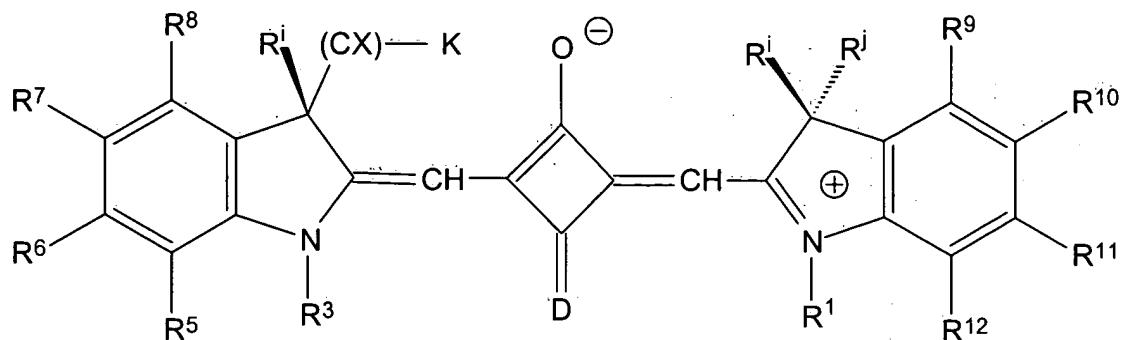
substituents capable of increasing the hydrophilicity of the entire compound, parts of a condensed aromatic or heterocyclic ring, and parts of a substituted condensed aromatic or heterocyclic ring; and

each H may be independently replaced by a fluorine.

5

33. The composition of claim 32, where at least one of Rⁱ and R^j is a reactive aliphatic group.

34. The composition of claim 32, wherein the composition has the formula



5 where D is =O, =S, =Se, =Te, =N-R^c, or =C(R^f)(R^g);

R¹ and R³ are independently H, -(CH₂)_k-L, or -(CF₂)_k-L where k = 1 - 30, and L

is one of H, F, Cl, Br, I, CH₂-NH₂, SO₃⁻, COOH, and CO-NHS;

R⁵ - R¹² are each independently H, F, SO₃⁻, PO₃²⁻, O-PO₃²⁻, PO₃R⁻, O-PO₃R⁻, -

(CH₂)_k-L, or -(CF₂)_k-L; where k = 1 - 30, and L is one of H, F, Cl, Br, I, CH₂-NH₂, SO₃⁻,

10 COOH, and CO-NHS F, or SO₃⁻, PO₃²⁻, O-PO₃²⁻, PO₃R⁻, or O-PO₃R⁻;

Rⁱ and R^j are H, aliphatic groups, alicyclic groups, aromatic groups, polyethers, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents and spacers containing one or more ionic substituents, capable of increasing the hydrophilicity of the entire compound; or Rⁱ and R^j taken in combination for a ring-system that is optionally further substituted one or more time by reactive or ionic substituents;

(CX) is an alkyl chain with 1-22 carbon atoms, a polyether chain, any other polycarbon chain, or a part of a ring system; and

K is COOH, N-hydroxy succinimide, iodoacetamide, maleimide, sulfonylchloride, phosphoramidite, SO_3^- , PO_3^{2-} , O-PO_3^{2-} , OH, or NH₂.

35. The composition of claim 32, wherein Z is based on squaric acid, croconic
5 acid, or rhodizonic acid.

36. The composition of claim 32, wherein at least one of Rⁱ and R^j includes a reactive group selected for reacting with amine moieties from the group consisting of N-hydroxysuccinimidyl esters, isothiocyanates, and sulfonylhalogenides.

10
37. The composition of claim 32, wherein at least one of Rⁱ and R^j includes a reactive group selected for reacting with thiol moieties from the group consisting of iodoacetamides and maleimides.

15
38. The composition of claim 32, wherein at least one of Rⁱ and R^j includes a reactive group selected for reacting with nucleic acids from the group consisting of phosphoramidites.

20
39. The composition of claim 32, wherein at least one of Rⁱ and R^j includes a linked carrier.

40. The composition of claim 39, wherein the carrier is selected from the group consisting of polypeptides, polynucleotides, beads, microplate well surfaces, and metallic nanoparticles.

5 41. The composition of claim 39, wherein the carrier is a polypeptide or a polynucleotide.

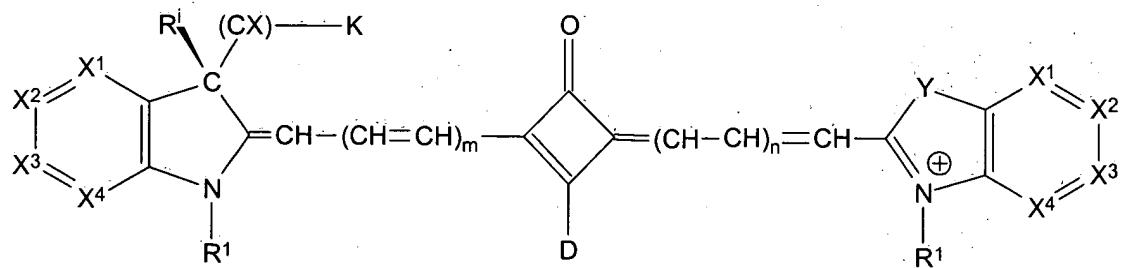
10 42. The composition of claim 32, wherein at least one substituent of Z includes an ionic substituent selected from the group consisting of SO_3^- , COO^- , PO_3^{2-} , O-PO_3^{2-} , PO_3R^- , $\text{O-PO}_3\text{R}^-$ and $\text{N}(\text{R}')_3^+$, wherein R and R' are aliphatic or aromatic moieties.

15 43. The composition of claim 32, wherein the photoluminescent compound is capable of covalently reacting with at least one of biological cells, DNA, lipids, nucleotides, polymers, proteins, and pharmacological agents.

20 44. The composition of claim 32, wherein the photoluminescent compound is covalently or noncovalently associated with at least one of biological cells, DNA, lipids, nucleotides, polymers, proteins, and pharmacological agents.

45. The composition of claim 32, wherein m and n are 1.
46. The composition of claim 32, further comprising a second reporter compound selected from the group consisting of luminophores and chromophores,
5 where the first reporter compound is an energy transfer acceptor and the second reporter compound is a corresponding energy transfer donor.

47. A compound having the formula



- 5 wherein D is selected from the group consisting of O^- , S^- , Se^- , Te^- , $N-(R^c)^-$, and $C(R^f)(R^g)^-$, wherein R^c is selected from the group consisting of aliphatic, heteroatom-substituted aliphatic, polyether, aromatic, reactive aliphatic, and reactive aromatic groups, R^f and R^g are selected from the group consisting of carboxylic acid, cyano, carboxamide, carboxylic ester, and aliphatic amine groups;
- 10 m and n are independently selected from the group consisting of 0, 1, and 2; Y is selected from the group consisting of O, S, Se, Te, $N-R^h$, and $C(R^i)(R^j)$, wherein R^h is selected from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, and reactive aliphatic groups, and wherein each of R^i and R^j are H, aliphatic groups, alicyclic groups, aromatic groups, polyethers, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, ionic substituents and spacers containing one or more ionic substituents, capable of increasing the hydrophilicity of the entire compound; or R^i and R^j taken in combination form a ring-system that is optionally substituted by one or more reactive or ionic substituents;

(CX) is an alkyl chain with 1-22 carbon atoms, a polyether chain, any other polycarbon chain, or part of a ring system;

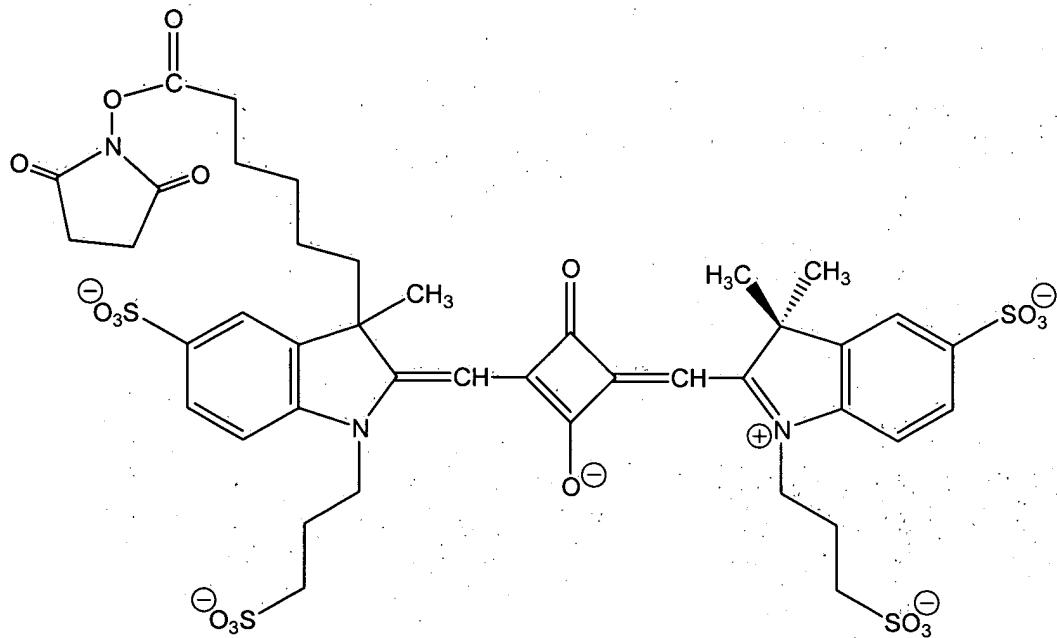
K is selected from the group consisting of COOH, N-hydroxy succinimide, iodoacetamide, maleimide, sulfonylchloride, phosphoramidite, and SO₃⁻, PO₃⁻, OH, or
5 NH₂;

each R¹ is independently selected for each of B and C from the group consisting of H, aliphatic groups, alicyclic groups, aromatic groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive groups capable of covalent attachment to a carrier, and ionic substituents capable of
10 increasing the hydrophilicity of the entire compound;

each of X¹, X², X³, and X⁴ is independently selected from the group consisting of H, N, O, S, and C-R^k, wherein R^k is selected from the group consisting of H, F, Cl, Br, I, aliphatic groups, alicyclic groups, aromatic groups, linked carriers, reactive groups capable of covalent attachment to a carrier, spacers bound to one or more reactive
15 groups capable of covalent attachment to a carrier, ionic substituents capable of increasing the hydrophilicity of the entire compound, parts of a condensed aromatic or heterocyclic ring, and parts of a substituted condensed aromatic or heterocyclic ring; and

each H may be independently replaced by a fluorine.

48. A compound having the formula



5 49. A method of performing a photoluminescence assay, the method comprising:

selecting a photoluminescent compound according to claims 32-48;
exciting the photoluminescent compound with excitation light; and
detecting emission light emitted by the photoluminescent compound.

10

50. The method of claim 49, including the step of detecting fluorescence.

51. The method of claim 49, including the step of detecting phosphorescence.